

Poster Presentation

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## The first auxotrophic mutant of *Zygosaccharomyces bailii* for recombinant productions: a road to practical applications

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from The 4th Recombinant Protein Production Meeting: a comparative view on host physiology  
Barcelona, Spain. 21–23 September 2006

Published: 10 October 2006

*Microbial Cell Factories* 2006, **5**(Suppl 1):P67 doi:10.1186/1475-2859-5-S1-P67

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### Background

The yeast *Zygosaccharomyces bailii* belongs to the so-called group of non-conventional yeasts, poorly studied in the past. For this reason, up to now there is no deep knowledge regarding its physiology, genetics and molecular biology, and its genetic manipulation is not easy. Only recently this yeast attracted the attention of the scientific community due to its characteristics of stress resistance, particularly to acidic environments. Our group is working on the construction and improvement of molecular tools for an exploitation of *Z. bailii* as a new host system for biotechnological applications.

### Results

In spite of the good results already obtained for the production and secretion of different proteins, a great limit was represented by the lack of an auxotrophic mutant and of a reproducible protocol for targeted gene deletion in this yeast. Here we show the strategy used to obtain the first *Z. bailii* auxotrophic mutant (*Zbleu2* strain) and the consequent exploitation of said mutant for heterologous protein production and for metabolic engineering applications. The data obtained with the new strain showed a great improvement of production, mainly related to higher plasmid stability, if compared with the wild type strain transformed with similar plasmid but selecting for an antibiotic resistance. In addition, and once more exploiting the *leu2* auxotrophy, we are developing a strategy for high copy number replication of an heterologous gene of interest.

### Conclusion

According to our knowledge, this is the first example of an auxotrophic *Z. bailii* strain exploited for recombinant productions. It represents a small but necessary step to develop this host for biotechnological applications, that already resulted in significant improvement in the productions of interest.